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The occurrence of pathogenic *E. Coli* in some types of soft cheeses in the local market

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ABSTRACT

The purposes of this practical research to detect the incidence of pathogenic E. *coli* in Kariesh, Domiatti And Tallaga cheeses. Confirmative serological identifications of the isolated strains of pathogenic *E. coli* were performed. One hundred and fifty random samples of Kariesh, Domiatti and Tallaga cheeses (50 each) were collected from different shops and street vendors in El Gharbia territories. Samples were bacteriologically examined for the presence of E. *coli*. The incidence of *E. coli* in the analyzed samples were 54%, 42%and 32%, respectively. Serotyping of isolated pathogenic serovars of *E. Coli* were: O119, O111, O86 & O128 and O124 in Domiatti cheese. Genotypic identification of *E. coli* virulence genes (Stx1, eaeA and fimH) were recorded at percentage of Zero %,28.5%and 100 %respectively. In Kariesh cheese. The detected E. *coli* in the analyzed samples surely able to causes a public health disease outbreaks to the consumer and stipulated the local hygienists to insures maximum health's stipulations. Therefore, this work was planned to cover the followings points:1-Isolations, biochemical and serological identifications of pathological Escherichia coli from kariesh, tallaga and domiatti types of cheeses in El _Gharbia governorate. Two Molecular identifications of isolated bacteria (bacterium coli) using PCR technique

1. INTRODUCTION

Among the dairy products, cheese is considered one of the most public sources of vital nutrients (*e.g.* vitamins, minerals and proteins) which represent the main part of healthy food (López-Expósito *et al.*, 2012). Nevertheless, cheese is a popular food for human being, sometimes its deterioration by various types of foodborne pathogens may take place. Manufacturing of cheeses is profitable due to high demands of consumers and low industrial risks, and there are many factories and farmers manufacturing it without supervisions and without health licenses (Koustaet al., 2010; Dias et al., 2012). In Egypt, kariesh cheese is a prevalent type of cheese which contains a higher protein content with a small amount of fats (Hamad, 2015), . and is of low prices so has poor's demands on a large scales in Egyptian cities and Arabian countries, similar to Domiati (Abd-Hamid, 2012). Domiatti cheese is one of soft pickled cheese produced and consumed on a wide scales due to rich in basic supplemental elements and had a pleasant taste, strong sharp flavor as well as smooth body and texture. Tallaga cheese is widely manufactured soft cheeses in El-Gharbia territories due to its values, with nearly neutral salty taste for hypertensive peoples of all ages and smooth textures for old peoples have teeth problems .

Escherichia coli is one of a genus of family Enterobacteriaceae of all warm-blooded animals, so confirmative detections constitutes the absences of health supervisions and the lack of medical bacteriological standards (Koustaet al., 2010; Dias et al., 2012). Enteropathogenic *E. coli* (EPEC) are considered the most common bacteria isolated from cheese. that cause high morbidity and mortality rates among young and old people (Koust*et al*., 2010). *E. coli* is environmental resistant fecal and urinal indicators, which has a bad health impacts on both human and animals' species. Thus, this type of bacteria can deteriorate the milk particularly raw milk and other milk products as a result of poor hygienic measures (Garbaj *et al*., 2016; Lara *et al*., 2016). Shiga toxin producing E. coli (STEC)have potent two exocytotic toxins which destructed all functional epitheliums causing per acute inflammations, bloody diarrhea, hemolytic uremic syndrome, encephalitis's and deaths (Pennington, 2010). STEC strains is world public hazard pathogen have two genes Stx1 and Stx2 may with each other or separately but constitutes powerful diseased virulence's genes and surely causing shigellosis similar diseases especially in weakly or hypo immunized individuals suffering from chronic diseases or viral diseases aging consumers (Lindgren et al., 1993; Muniesa et al., 2004).

STEC detected serotypes and genotypes is surely classified food-borne pathogens because of destructions of all parenchymatous organs causing breakdowns, suffering and outbreaks of deaths. (Bach et al., 2002; Blanco et al., 2003). The most common serovars worldwide were O26, O91,

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O103, O111, O128, O113 and O145 are responsible for up to 20 to 50% of all STEC infections (Johnson et al., 2006). The somatic serovars causing seriously humans' chronic diseases in the developing countries were 26, 91, 103, 111, 113, 121, 145 and 157 (Karmali et al., 2010; Pizarro et al., 2014). Eae A gene it is an intimin secretion gene that enhance attachment and colonization's of Escherichia coli in the intestinal epithelium leading to sever cellular destruction and severe infections and sever symptoms like profuse diarrhea and ulcerative colitis (Ibrahim et al., 2019).Urinary specific Escherichia coli is genetically based on the fimbrial structural type 1 gene which responsible for most UTIs diseased cases. Uro-pathogenic E. coli (UPEC) strains is the hidden causative bacteria of vaginitis and causes vaginal hemorrhage especially in females based on honest experimental results whom real medical scientists. The most virulence factor dependent upon the UPEC include adhesions type 1 fimbriae encoding by fim H gene (Zohreh et al., 2014).

2. MATERIAL AND METHODS

2.1. Sampling:

One hundred and fifty random samples of soft cheese (50 samples of each of kariesh, domiatti and tallaga cheeses) were collected randomly from small diaries, local supermarkets, farmers house, shops and street vendors in different localities at El-Gharbia Governorate, Egypt. The selected samples were delivered directly to the institute in an ice – box under aseptic conditions without delay and then examined bacteriologically.

The bacteriological examinations of samples were carried out in Animal Health Research Institute Tanta, while serological examinations were done in Animal Health Research Institute Dokki.

2.2. Bacteriological examination (Virpari et al., 2013; Lofty et al., 2017; Ibrahim et al. et al. 2020)

2.2.1. Preparation of samples:

Five grams of each cheese sample were added to 25 ml sod. Citrate and blended for two minutes before examination.

2.2.2. Isolation of E. coli:

Two ml of each prepared sample are added to MacConkey broth tubes and incubated for 24hours at 37ºC for the detection of acid and gas as indicative for the presence of *E. coli.*

From the positive MacConkey's broth test with yellow colorations (acid) and gas results are cultured on the Eosin Methylene Blue (EMB) agar plates, as a selective media for *E. coli*, then incubated at 38 ºC for 24 hours. Typical colonies of *E. coli* appeared greenish metallic with dark purple center. Suspected colonies were detached by bacteriological loop and transferred to nutrient agar slopes and then incubated at 38 ºC for 24 hours. The purified colonies were subjected for further morphological, biochemical and serological examination.

2.3. Identification of isolates:

2.3.1. Morphological identification: E. coli appear as Gram negative bacilli arranged singly or in short chains.

2.3.2. Biochemical identification:

The pure colonies of isolates were identified biochemically as follow: Indole, Methyl red, Vogues-Proskauer, Simon citrate and Triple sugar iron tests were done. Results were recorded according to the following information

Bacteria	Biochemical tests				
E. coli	Indole	Methyl red	Voges–Proskauer	Citrate utilization	TSI
	+	+	-	-	A/A -ve H_2S

2.3.3. Serological identification of E. coli sero vars:

Slide agglutination test were done with suspected colonies with poly valent antisera if positive for agglutination this colonies is confirmative positive for Escherichia coli and the same test were done for positive one with monovalent antisera to detect the somatic antigen because each strain contains only one somatic antigen in their body.

2.4. In-Vitro antibiotic sensitivity of isolates.

Subcultures from the isolates were prepared and the test was applied as follows: A smooth single colony was inoculated in 5 ml nutrient broth and incubated at 37°C for 18 hrs., then turbidity was adjusted to 0.5McFarland contain (1.5×10^8) colony forming unit/ml, then few drops of the inoculated broth were flooded on to the surface of Muller-Hinton agar plates. Excess of cultural fluid was removed aseptically, and the plates were allowed to stand for 15 minutes at 37°C for dryness. Then the inoculated plates were over laid with antibiotic discs using sterile forceps, the discs were distributed in a manner where the distance among them was optimum and away from the edge of plate to avoid overlapping of inhibition zones and give more wide area for the zone of inhibition. The inoculated plates were incubated at 37°C for 24 hours. The Inhibition zones, in mm were measured and scored as sensitive, intermediate and resistant categories with the critical break points recommends by CLSI (CLSI, 2018) as shown in table (1).

Table 1 Antimicrobial discs, concentration or antibiogram profile of antibiotic susceptibility

Susceptible (mm)	Intermediate (mm)	Resistant (mm)	Sensitivity disc content (µg)	Antimicrobial agent
18 or more	13-17	12 or less	30	Chloramphenicol (C)
20 or more	15-19	15 or less	5	Ciprofloxacin (CP)
23 or more	14-22	13 or less	15	Erythromycin (E)
4 or more	1-2 0	,5 or less	5	Enrofloxacin (ENR)
17 or more	13-16	12 or less	30	Neomycin (N)
128 more	64	32 or less	300	Nitrofurantoin (F)
19 or more	15-18	14 or less	30	Oxytetracycline (OT)

2.5. Detection of virulence factors of E. coli:

Extraction of DNA according to QIA amp DNA mini kit instructions, Preparation of PCR Master Mix According to Emerald Amp GT PCR master mix (Takara) Code No. RR310Akit, Cycling conditions of the primers during c PCR (temperature and time conditions of the two primers during PCR according to specific authors and Emerald Amp GT PCR master mix (Takara kit) they have specific sequence and amplify a specific product as shown in table (2), DNA Molecular weight marker the ladder 100 bp was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded and agarose gel electrophoreses (Sambrook *et al*., 1989).

Table 2 Oligonucleotide primers sequences Source: Metabolon (Germany).

Gene *E. coli*	Sequence	Amplified product	Reference
Stx1	ACACTGGATGATCTCAGTGG	614 bp	Dipinto *et al.* (2006)
	CTGAATCCCCCTCCATTATG		
fimH	TGCAGAACGGATAAGCCGTGG	508 bp	Gangapur and Salehi (2010)
	GCAGTCACCTGCCCTCCGGTA		
eaeA	ATGCTTAGTGCTGGTTTAGG	248 bp	Bisa-Johnson *et al*. (2011)
	GCCTTCATCATTTCGCTTTC		

3. RESULTS

Sixty-four of suspected selective bacterial recognitions were obtained from examined samples, of them 27 from karieshcheese,21 from domiatti cheese. and 16 from tallaga cheese. *E*. coli isolated from kariesh, domiatti and tallaga cheeses with an incidence of54%, 42 and 32 respectively as shown in Table 3.

Serological identification revealed that five isolates from kariesh cheese were: (O119, O111, O86: O18 and O128). One isolates from domiatti cheese was: (O124) (Table 4).

We choose a reasonable number to confirm that culturing on (EMB) is somewhat trusted because it is the main dean of isolations of the chosen a risk food borne pathogens.

4. DISCUSSION

Illness caused by entero-pathogenic *E. coli* can range from self-limited watery diarrhea to life threatening manifestations such as hemorrhagic colitis, hemolytic uremic syndrome, thrombocytopenic purpura and may lead to death (Alexander and Prado, 2003). Recently there is additional concern about the presence of *E. coli* in cheeses because of the ability of some strains to cause food borne illness and lead to high incidence of food outbreaks.

Table 3 Incidence of *E. coli* isolated from soft cheeses samples:

Examined samples	E. coli positive *samples*	
	%	*number*
Total cheese n=150	42.5	64
Kariesh cheese n=50	54	27
Domiatti cheese n=50	42	21
Tallaga cheese n=50	32	16

Table 4 Results of serological identification of *E*. coli isolates.

Serial	Identified Bacterium	Serodiagnosis	Strain
1	*E*. coli(K3)	O119: H7	EPEC
2	*E*. coli(K1)	O111: H8	EHEC
3	*E*.coli (K2)	O86	EPEC
4	*E*. coli(K5)	O18:H7	Ex PEC
5	*E*. coli(K6)	O128	EIEC
6	*E*. coli(D3)	O124	EPEC

K =Kariesh cheese, D=Domiatti cheese, T= Tallaga cheese.

Table 5 Antimicrobials sensitivity results for *E.* coli isolates

Antimicrobial agent	Diffusion zone break point (Mm)	E. coli D3 O124	E. coli K3O119	E. coli K2 O86	E. coli K1 O111	E. coli K5O18	Sensitivity Percent
Ciprofloxacin (CIP)	12 (mm)	S (20)	S (28)	S (29)	S (27)	S (32)	100%
Enrofloxacin (ENR)	0.5(mm)	S (8)	S (9)	R (0.3)	S (7)	S (8)	83.33%
Chloramphenicol (C)	15(mm)	S (23)	S (26)	R (7)	R (8)	I (17)	59%
Oxytetracycline (OT)	14(mm)	S (22)	S (21)	R (11)	S (25)	S (24)	83.33%
Neomycin (N)	12(mm)	I (14)	R (9)	R (8)	R (9)	R (7)	%8
Nitrofurantoin (F)	32(mm)	R (25)	S (145)	R (24)	R (19)	S (149)	50%
Erythromycin (E)	13(mm)	R (9)	I (17)	R (9)	S (28)	R (7)	25%

S= sensitive. R= Resistant. I = intermediate. K= Kariesh cheese. D= Domiatti cheese

Table 6 Results of molecular identification of *Stx1, eae A* and *fim H* genes of *E.* coli isolates.

Results			Target MO
STX1	eae A	fim H	isolate
-ve	-ve	+ve	1
-ve	-ve	+ve	2
-ve	+ve	+ve	3
-ve	+ve	+ve	4
-ve	-ve	+ve	5
-ve	-ve	+ve	6
-ve	-ve	+ve	7

E. Coli

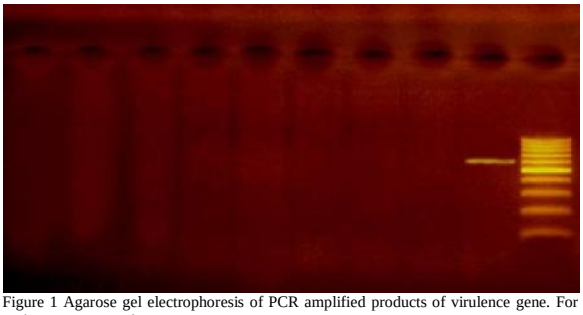

Figure 1 Agarose gel electrophoresis of PCR amplified products of virulence gene. For stx1 gene was negative.

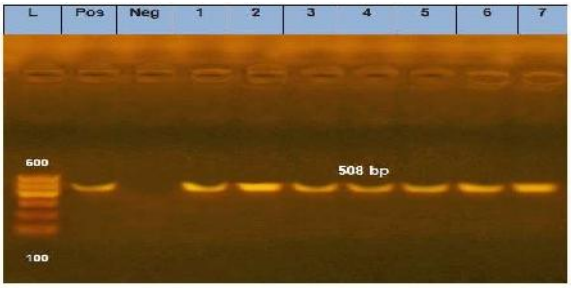

Figure 2 Agarose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 1,2,3,4,5,6,7: fim H virulence gene of E. coli. The size in base pairs (508bP) of PCR product is indicated for the bands.

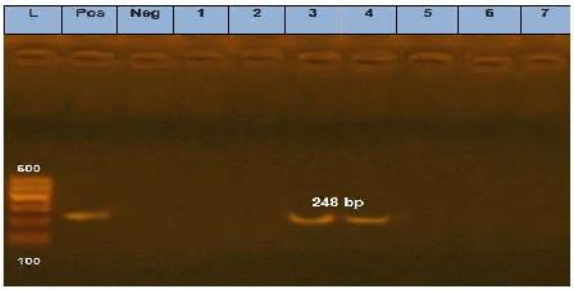

Figure 3 Agarose gel electrophoresis of PCR amplified products of virulence gene. Lane L: DNA molecular size marker (100bP), lane Pos: Positive control, lane Neg: Negative control, Lane 2,3: *eae A* virulence gene of *E.* coli. The size in base pairs (248bP) of PCR product is indicated for the bands.

Inspection of table 3 revealed that E. coli could be isolated from examined kareish cheese samples with incidence rate of 54%. Nearly similar finding was reported by Nagah et al. (2012), El-bajory et al. (2015) and El-nahas et al. (2015). Higher incidence was reported by Ibrahim et al. (2019), Ombark et al. (2016), El-kosi (2001), while lower incidence was reported by El-Sayed et al. (2011), Hosney et al. (2011), Ahmed (2012), Brooks et al. (2012), Nagah et al. (2012), Elhadidy and Mohammed (2013), Bonyadian et al. (2014), Abo Zeed (2014), Metwally and Fatma (2015), El-nahas et al. (2015), Gamal et al. (2 015), Jehan et al. (2015), Gamal and Soad (2016), and Awad (2016). It is evident that E. coli could be isolated from examined Damietta cheese samples with incidence rate of 42%. Nearly similar finding was reported by Ozen et al. (2011), Ibrahim et al. (2019), while lower incidence was reported by Nagah et al. (2012), Elhadidy and Mohammed (2013), Gamal and Soad (2016), Jehan et al. (2015), Gamal et al. (2015), Elbassary (2006) and El-bajory et al. (2015).

It is evident from table 4 that E. coli could be isolated from examined tallaga cheese samples with incidence rate of 32%. Nearly similar finding was reported by El-Sayed et al. (2011), while lower incidence was reported by Ahmed (2012).

Contamination of milk and milk products, with E. coli is largely due to unhygienic condition during production, processing, handling, and distribution. E. coli is a good indicator of fecal pollution as it exists in the normal microflora of the intestinal tract of humans and warm-blooded animals (Virpari et al., 2013; Lofty et al. 2017; Ibrahim et al., 2020). It is evident from the results recorded that some of E. Coli isolates from some types of soft cheeses that could be identified serologically were (O111) (EPEC) Enteropathogenic E. coli (O86) (EHEC) Enterohemorrhagic E. coli, (O18) (ExPEC) Extera intestinal pathogenic E. coli, (O128) Atypical EPEC E. coli, (O124) (EIEC) Entero-Invasive E. coli. CLSI :(2018).

Isolates show sensitivity to the antibiotics used in this test as 100% for Ciprofloxacin CIP, 83.3% for Enrofloxacin, 83.3% for Oxytetracycline, 50 % for Chloramphenicol, 50 % for Nitrofurantoin, 8% for Neomycin and 25% for Erythromycin. The common mistakes of antibiotics in the veterinary field in treating of colibacillosis in animals or coli septicemia in humans is the main cause selecting the resistance of pathogenic types of Escherichia coli as the selected antibiotics not the most killer one .

Stx1 gene is Shiga toxin potent exocytotic and cause severe illness not found in all E. coli isolates. Nearly similar findings were reported by Taha et al. (2019), while higher incidences findings were reported by Sabry et al. (2008), El-hadidy and Mohamed (2012), Ombark et al. (2016), Cholesteric et al. (2017), Ibrahim et al. (2019) and Hussein et al. (2019).

Eae A gene is an intimin secretion gene that enhance attachment and colonization's, it was detected in our E coli isolates by PCR at percentage of 28.5 % while lower incidences findings were reported by Ibrahim et al., (2019), Sabrey et al. (2008), Hussien et al. (2019), El-hadidy and Mohamed (2012), and Chalechtori et al. (2017).

FimH gene is responsible for biofilm formation and enhance infection of Uro-pathogenic Escherichia coli and the structural rule of type 1 fimbriae and responsible of about 92 % of the pathogenicity and diseased cases in human patients suffering from Urinary tract infections (Garofalo et al., 2007). Its presence in our isolates by PCR was 100% of E. coli isolates. Similar results and findings were reported by Garofalo et al. (2007) while lower

incidences findings were reported by Sabrey et al. (2008), Mladin et al. (2009), Arabi et al. (2012), Zohreh et al. (2014), and Hussien et al. (2019). According to Egyptian standard (2005) milk and milk products must be free from E. coli.

5. CONCULOSIONS

Practical bacteriological examinations executed in animal health institute revealed that the soft cheeses ready for sales and consumptions exhibited in the market of El Gharbia governorate show high Escherichia coli incidence due to weak or absence of responsible authorities. Milk used for cheese manufacture must of good qualities and from dairy cattle's free from mastitis, farmers manufactured cheeses must free from diseases especially Escherichia coli infections all equipment's must be cleaned and sanitized by veridical, bactericidal and fungicidal detergents. Finally, the present study allows to conclude that kariesh cheeses more contaminated with pathogenic Escherichia coli with heath fatal or chronic complications likes hemorrhagic colitis, hemolytic uremic syndrome and meningo-encephalitis and even death. The serotyping confirmed the present of more virulence serotyping worldwide as O 111, The best antibiotics in treatment to saves life's were detected as follow ciprofloxacin , enrofloxacin and oxytetracycline , so strict hygienic measures during the production , transportation and selling of un pasteurized kariesh cheeses and regulatory checking of the workers is of great importance to prevent the contaminations with Escherichia coli.

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